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Optical Immunoassay for Streptococcal Pharyngitis: Evaluation of Accuracy with Routine and Mucoid Strains Associated with Acute Rheumatic Fever Outbreak in the Intermountain Area of the United States

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The Strep A OIA (BioStar, Inc., Boulder, Colo.) rapid detection system is an intriguing technology that utilizes an immunoassay relying on changes in reflected light to directly detect group A streptococcal antigen from specimens. In this evaluation, 424 routine pediatric throat specimens and 20 simulated oropharyngeal specimens with added mucoid (M type 3, 18) strains were cultured and tested by the Strep A OIA. The respective sensitivities and specificities were as follows: Strep A OIA versus enhanced broth culturing, 84.2 and 95.7%; and streptococcus-SXT agar (BBL Microbiology Systems, Cockeysville, Md.) culturing versus enhanced broth culturing, 82.9 and 98.6%. The Strep A OIA is an 8-min, technologist-friendly, accurate technique with an 89.4% agreement with traditional culturing.

Streptococcus pyogenes (a group A streptococcus) has recently been the focus of intense clinical interest in the Intermountain West because of an outbreak of rheumatic fever (6) and the occurrence of toxic shock-like syndrome (5). Because a large majority of the community physicians utilize antigen detection technology to diagnose group A streptococcal pharyngitis, we evaluated the sensitivity of Strep A OIA (BioStar, Inc., Boulder, Colo.) for the detection of routine and mucoid (M type 3, 18) strains of group A streptococci from either simulated pharyngeal specimens or actual specimens obtained from children with pharyngitis.

Specimen collection and culturing. The Strep A OIA test was compared with streptococcus-SXT agar (BBL Microbiology Systems, Cockeysville, Md.) and enriched broth (Todd-Hewitt; BBL Microbiology Systems) as previously described (3). In brief, oropharyngeal swab specimens were collected from patients with pharyngitis by a single-swab technique. Synthetic-fiber throat swab (Culterette system; BBL) specimens were obtained or were simulated to contain various amounts of mucoid group A streptococci and normal pharyngeal flora according to previously published procedures and submitted to the laboratory. In brief, plating of a sterile swab immersed in saline dilutions and then thoroughly moistened in fresh saliva would result in 1+ (less than 10 CFU), 2+ (10 to 30 CFU), and 4+ (growth in the fourth quadrant) readings. Trypticase soy agar plates containing 5% sheep blood, sulfamethoxazole, and trimethoprim were inoculated, streaked for isolation, stabbed to introduce an inoculum below the agar surface, incubated at 35°C in a CO_2 incubator, and read at 24 and 48 h. Colonies which were beta-hemolytic, inhibited by an 0.04-U bacitracin disk (BBL), and positive for the enzyme pyroglutamyl aminopeptidase (Strep-A-Chek; E-Y Laboratories, San Mateo, Calif.) were presumptively identified as group A streptococci (2) and serotyped by the enzyme extraction technique (PathoDx Strep Grouping Kit; Diagnostic Products Corp., Los Angeles, Calif.). The swab was then used to perform the Strep A OIA procedure following the manufacturer's package insert instructions as described below. In addition, the pledgets (plugs separating the media and swabs in the transport tubes) were placed in Todd-Hewitt broth and incubated for 24 h at 35°C. All specimens were then subcultured to the same plates as the primary culture, incubated, and read at 24 and 48 h.

Strep A OIA technique. Following the manufacturer's instructions, the swab was extracted in 0.3 M acetic acid (reagent 1) for 2 min. This reaction was neutralized (reagent 2), and the labeled antibody was added (reagent 3). The sample was subsequently placed on the OIA membrane for a 2-min incubation. The next step was a rinse cycle (reagent 4). The final reagent was a substrate (reagent 5) which was added and then incubated for 4 min. After one more rinse (reagent 4), a color change to purple was interpreted as positive, and a gold color, with a procedure control dot, was interpreted as negative.

Routine specimens. A total of 424 routine throat culture samples were evaluated. Table 1 summarizes the performance characteristics of the Strep A OIA test and streptococcus-SXT agar culturing versus enhanced broth culturing. Enhanced broth culturing resulted in 76 positive specimens, Strep A OIA resulted in 79 positive specimens, and streptococcus-SXT agar culturing resulted in 68 positive specimens. Compared with the enhanced broth technique, Strep A OIA had a sensitivity of 84.2% and a specificity of 95.7%, with the three additionally positive specimens that were detected by Strep A OIA not being detected on streptococcus-SXT agar. Compared with the enhanced broth technique, the streptococcus-SXT agar technique had a sensitivity of 82.9% and a specificity of 98.6%. The agreement (percent agreement was calculated by dividing the sum of the true-positive and true-negative results by the total number of specimens)

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TABLE 1. Comparison of enhanced broth culturing with Strep A OIA and streptococcus-SXT agar culturing for detection of group A streptococci

Enhanced broth culturing result	No. of specimens with the indicated result in:			
	OIA test[a]		Streptococcus-SXT agar culturing[b]	
	Positive	Negative	Positive	Negative
Positive	64	12	63	13
Negative	15	333	5	343

[a] In comparison with enhanced broth culturing, OIA had 84.2% sensitivity, 95.7% specificity, a positive predictive value of 81.0%, and a negative predictive value of 96.5%.

[b] In comparison with enhanced broth culturing, streptococcus-SXT agar culturing had 82.9% sensitivity, 98.6% specificity, a positive predictive value of 92.6%, and a negative predictive value of 96.3%.

between Strep A OIA and streptococcus-SXT agar culturing was 89.4%.

Mucoid strains. Of the 20 group A streptococcal strains shown to be mucoid by culturing, all were detected by Strep A OIA.

An increase in the incidence of acute rheumatic fever in the United States has understandably received much attention. The reported outbreaks, which occurred primarily between 1984 and 1987, represent a notable departure from historic trends in acute rheumatic fever incidence and demographics. In addition, recent reports suggest an increase in severe and invasive group A streptococcal infections, perhaps indicating an increase in virulence among this group of organisms (1).

In this evaluation, Strep A OIA was found to be 84.2% sensitive and 95.7% specific when compared with enhanced broth culturing. Indeed, Strep A OIA is more sensitive than streptococcus-SXT agar culturing (82.9% sensitivity) when both are compared with enhanced broth culturing. Furthermore, in this evaluation, the agreement between traditional culturing and Strep A OIA was 89.4% for this patient population.

The advantage of Strep A OIA is the speed with which a diagnosis of group A streptococcal pharyngitis can be confirmed with a high degree of specificity (95.7%). Strep A OIA yielded complete agreement with culturing for the detection of mucoid group A streptococci from simulated throat swabs. As reported in this study, the Strep A OIA optical immunoassay can be successfully used for the direct detection of mucoid strains of group A streptococci from 2-min extracts of throat swabs.

Because of the excellent turnaround time (8 min) and simplicity of Strep A OIA, this test has potential for being a significant advance in diagnostic testing of pediatric specimens (4). Coupled with the admirable performance characteristics of sensitivities above those of traditional culturing techniques in comparison with enhanced broth culturing, Strep A OIA should have a significant role in streptococcal pharyngitis diagnosis.

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